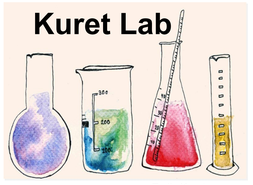

A Tool for Diagnosing and Staging Synucleinopathies

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ABSTRACT

Synucleinopathies are neurodegenerative diseases characterized by the abnormal accumulation of α-synuclein protein aggregates in the brain. Parkinson's Disease, the leading movement disorder and synucleinopathy, encompasses roughly 9 million people worldwide and costs in the United States alone total $25 billion yearly. However, there are no standard diagnostic tests for a biological marker of Parkinson's, such as a blood test or imaging scan. Difficulty in designing an imaging agent stems from the challenges of crossing the blood brain barrier, binding selectively to authentic lesions, and remaining low risk for human patients. This study aims to create an imaging agent that can detect and stage α-synuclein distribution *in vivo* (live in the patient) via positron emission tomography. Using immunohistochemical methods, human tissue was stained using a commercially available polyclonal anti-α-synuclein antibody and imaged using a con-focal microscope. Tissue stained with our small molecules tagged with a fluorescent ligand recapitulated the images of the positive control, validating that our molecule bound to the target. Preliminary data have provided promising results of the development of an imaging agent that binds authentically to α-synuclein and has the characteristics necessary to pass the blood brain barrier while remaining safe for human consumption. To continue verification, further testing will be done against Tau and β-amyloid protein to ensure the molecule's selectivity for α-synuclein. Successful completion of this project will provide an objective diagnostic tool for physicians and patients. This tool will be critical in earlier diagnosis and staging of disease, aiding patients and healthcare providers towards better medical treatment and disease outcome.

INTRODUCTION

- PD is a major neurodegenerative disease of the elderly
- PD is accompanied by the accumulation of intracellular lesions in the brain
- These lesions, named Lewy Bodies, contain α-synuclein in an aggregated form (Fig. 1).
- As PD progresses, aggregate density increases and spreads in a stereotypical fashion (Fig. 2)
  - A third of cases go on to develop Lewy body dementia (LBD), characterized by cortical deposition of α-synuclein aggregates
- **PROBLEM**: Currently no pre-mortem biomarker for Lewy bodies exists.
  - **Biomarkers are needed for early detection, staging, and differential diagnosis of PD and especially LBD**

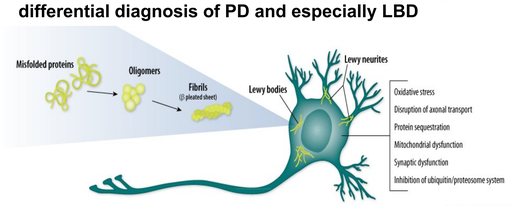

Figure 1: α-synuclein aggregation in neurons (R&D Systems, 2014).

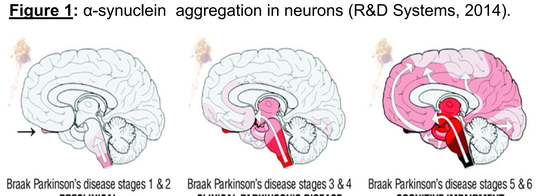

Figure 2: Progression of PD (Braak, 2002)

APPROACH

- **Hypothesis**: radio-labeled small-molecule probes capable of selectively binding α-synuclein aggregates could provide premortem assessment of synucleinopathies through whole brain imaging

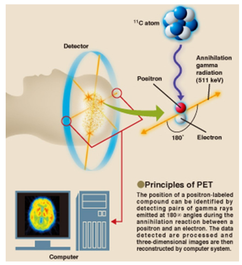

Figure 3: Positron emission tomography (PET). This whole-brain imaging technique can capture spatial distribution of diverse biomarkers.

- Barriers to progress
  - Probes must be α-synuclein selective
    - Confounding binding sites are present in Aβ and Tau aggregates
  - Probes must pass the blood brain barrier
    - clogP (hydrophobicity): 2-4
    - Topological polar surface area (tPSA): <70 Å²
  - Probes must have appropriate pharmacokinetics (PK)
    - Rapid uptake and wash out
- This project seeks to identify compounds that meet these requirements.
  - Identify candidate imaging agents
  - Histochemically stain human brain tissue
    - A commercially available antibody was used to stain PD tissue for comparison use with candidate molecules
    - PD tissue as positive control
    - AD tissue as negative control

RESULTS

1. Candidate small molecules express appropriate binding specificity, hydrophobicity and tPSA (Fig. 4)

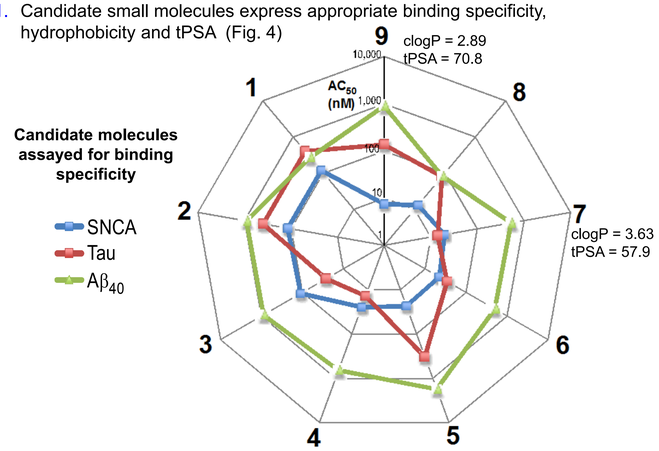

Figure 4: Polar plot (signal), where the spokes correspond to displacement AC50 in units of *nM*. As one moves from center to outer rings, affinity drops. Note that AC50 is not K_i. Divide numbers by ~2 for K_i. Compounds **1-9** synthesized, tested, and results ordered with respect to s[illegible]n (SNCA) displacement activity (blue). Aβ (green) is present at high concentrations in brain and obscures imaging of Tau and SNCA.

RESULTS (continued)

2. Tissue sections prepared from the substantia nigra region of authentic PD brain demonstrate Lewy bodies.(Fig. 5AB)
3. A fluorescent candidate small-molecule successfully stained Lewy bodies (Fig. 5C)

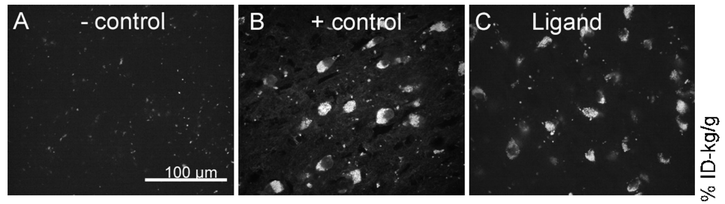

Figure 5*:* Candidate molecule stains PD brain lesions ***Parkinson's disease substantia nigra* examined by confocal fluorescence microscopy.** **A**, autofluorescence; **B,** 0.5 µg/ml commercially available sheep polyclonal antibody ab6162/Texas red-anti-sheep secondary antibody; **C,** ligand stain (100 µM). Panels **A** and **C** were captured at λ_{ex} = 515, λ_{em} = 550/49. Panel B was captured at λ_{ex} = 561, λ_{ex} = 625/90. Both antibody and ligand stain Lewy bodies in substantia nigra.

4. A fluorescent candidate small-molecule did not stain tau lesions (Fig. 6D)

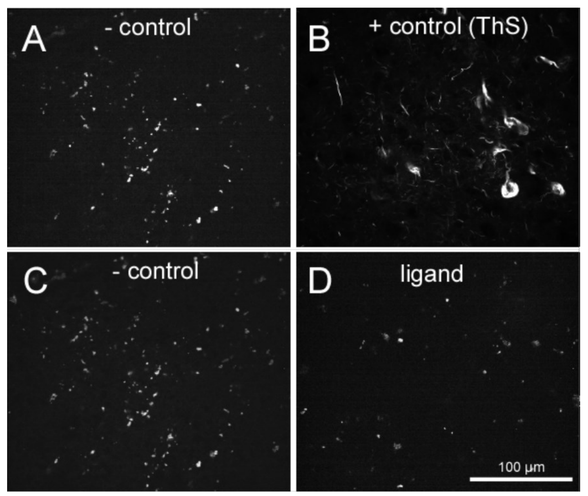

Figure 6*:* Candidate molecule does not stain AD brain lesions ***AD hippocampus* examined by confocal fluorescence microscopy.** **A**, autofluorescence; **B**, ThS stain. Both panels **A** and **B** were captured at λ_{ex} = 445, λ_{em} = 480/40. **C**, autofluorescence (same section as panel A, but captured at different wavelengths); **D**, ligand stain (100 µM). Both panels **C** and **D** were captured at λ_{ex} = 515, λ_{em} = 550/49. ThS, but not ligand, stains neurofibrillary pathology.

RESULTS (continued)

5. Preliminary data shows appropriate pharmacokinetics for rapid uptake and wash out (Fig. 7)

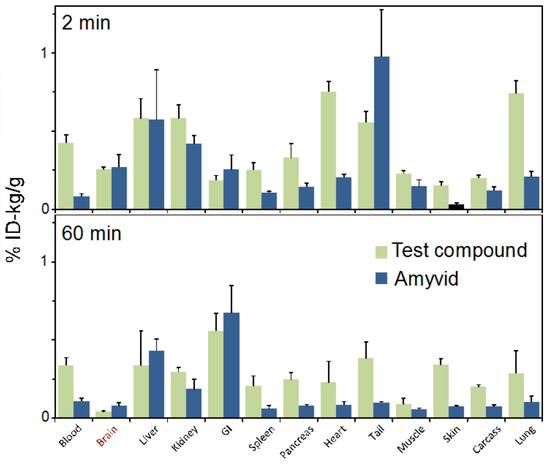

Figure 7: Preliminary PK data, rapid uptake/washout

DISCUSSION

- α-synuclein aggregates are a tractable target for small-molecule radiotracer development
  - Binds recombinant α-synuclein selectively over tau and Aβ
  - Binds authentic Lewy bodies in PD tissue
- Appropriate clogP, tPSA, and PK values suggest the scaffold class may have utility *in vivo*
- FUTURE STUDIES
  - Structure activity relationship analysis to identify lead molecule
  - Preparation of radiolabeled compounds for advanced preclinical investigation
    - Direct binding experiments
    - Distribution, metabolism, and pharmacokinetics

ACKNOWLEDGEMENTS

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